Environmental Health Perspectives
Vol. 19, pp. 173–177, 1977

Experimental Studies on Arsenic Absorption Routes in Rats

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Pentavalent inorganic arsenic was introduced by intravenous, intratracheal, gastrointestinal, and skin application in doses 0.1 to 4.0 mg/kg in rats. Isotopic technics were applied by use of As^{74}. It was found that the dynamics of arsenic distribution in the body as well as the kinetics of its elimination in urine and feces varies very substantially, depending on the mode of administration. Intravenous administration of As causes immediate appearance of arsenic in most tissues and a slow decrease of its concentrations in time. Similar situations could be observed with intratracheal dosing, because arsenic is very rapidly absorbed from the site of administration. Concentration in tissues increases more slowly after gastrointestinal resorption. Skin application causes first the accumulation of arsenic in the skin and next continuous, slow transport from the skin into the blood stream.

The rate of skin resorption was 1.14–33.1 $\mu g/cm^2$-hr for 0.01–0.2*M* concentrations. The red blood cell level of arsenic is very substantial and does not change with time, which indicates the accumulation of arsenic in this tissue.

The elimination of arsenic occurred chiefly in urine and feces, but the urine/feces ratio changed very substantially, depending on the route of administration. The kinetics of arsenic elimination in urine was multiphasic, being three-phase in case of intravenous and intratracheal administration and two-phase after gavage and skin resorption. After intravenous administration of As, the half-times of elimination were 2.5, 10, and 690 hr, respectively.

Administration of selenium salts during the slow phase increased the rate of arsenic elimination. The straight-line relations found between the absorbed dose of arsenic and its blood or urine concentrations could serve as baselines for exposure tests for humans.

Introduction

In industrialized regions, like upper Silesia in Poland, there arise many environmental toxicology problems. One of the most important is the evaluation of exposure of human populations to toxic metals. An example is the study of metal content in hair of school children in this region *(1)*. By use of a neutron activation method, 17 elements have been found, among others Zn, Cd, Cu, Hg, Se, and also arsenic, which appeared in higher concentrations in the vicinity of zinc smelters.

Arsenic is widely distributed in nature and its toxic properties have been known for centuries. Sometimes arsenic occurs in the lithosphere or in the hydrosphere in significant concentrations, and then this deserves special attention. Such is the case in a region in Czechoslovakia where coal containing up to 1530 mg/kg of arsenic compounds is used (*2*) or where well water, near an arsenic mine in Poland, has an arsenic concentration up to 0.33 mg/dm^3 (*3*). Burning of coal and some industrial procedures as smelting of arsenic-containing ores, agriculture use of arsenic fungicides and pesticides, and even household detergents can produce contamination of the environment with arsenic compounds.

From the Novaky power station in Czechoslovakia 138 tons of arsenic enter the atmosphere in one year (*4*).

In the soil, the arsenic concentrations can rise to hundreds of milligrams per kilogram (*5*). The presence of arsenic compounds in soil, water, and air leads to their intake in plants and animals, including man. In this point there arises the question of proper methods of evaluation of exposure of human populations. Both industrial and environmental exposure should be considered, as well as the main sources of absorption: air, water, and food.

In evaluation of exposure, it is most essential to know the absorbed dose during a defined period of

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time. This implies knowledge of uptake, distribution in the body, and elimination, taking into consideration all possible routes of absorption.

Very often concentrations of metals in index media (blood, urine) are taken as indicators of dose. To use the metal content in blood or urine as an indicator of exposure, the absorption–elimination or absorption–concentration relationship must be known. This implies the necessity of distribution dynamics and elimination kinetics studies by various ways of absorption.

No sufficient data were available concerning the behavior of arsenic inorganic compounds in the body, and for this reason the recent study was undertaken. It includes the absorption kinetics of arsenic by blood red cells *in vitro* and *in vivo*, the dynamics of arsenic distribution in the body, the kinetics of elimination of arsenic in urine, feces, and expired air, the distribution and elimination of arsenic when administered daily, and investigation of compounds influencing the elimination rates. Four routes were taken into consideration; intravenous and intratracheal administration, gastrointestinal (gavage), and skin resorption.

The results deal only with the differences or similarities in arsenic distribution in the body and the kinetics of elimination after various ways of absorption. Administration of selenium and its action on the elimination of arsenic will be also considered.

Methodology

The majority of experiments were performed on white 170–200 g female, Wistar rats, fed a standard diet. Drinking water was given *ad libitum*. During the experiments the animals were kept in all-glass metabolic Simax cages that allowed separate collection of urine and feces samples. The samples were taken each day.

Arsenic in the form of sodium arsenate $Na_2HAsO_4 \cdot 7 H_2O$ was used as a carrier and labeled with ^{74}As. Various doses of arsenic were used (in the range 0.1–4.0 mg/kg) but in all cases the single dose had a volume of 0.3 cm^3 and an activity of 1–2 μCi (160,000–320,000 counts/min).

In experiments of skin resorption of sodium arsenate aqueous solutions, the rats tails were immersed for 1 hr in 0.01, 0.1, or 0.2*M* solution.

The animals were sacrificed by desanguination under ethyl ether narcosis after 1, 2, 5, 24, 72, 120, 168, and 240 hr, and arsenic was determined in the following organs and tissues: bowels, muscles, bones, skin (skin of tails separately in skin resorption), liver, kidneys, lungs, spleen, heart, blood, and hair. The samples of feces, bowels, skin, muscles, and bones were homogenized, blood and urine were diluted with water, the remaining organs and tissues were digested by the use of sulfuric and nitric acid.

The content of arsenic was calculated by comparison of the measured activity of the sample with the activity of a standard solution.

Results and Discussion

The results presented actually in this paper are limited to those which illustrate the differences or similarities in various routes of application (*6*).

Arsenic content in various organs after intravenous application is given in Table 1. The higher concentrations were obtained in liver, kidneys, lung, spleen, and blood. The figures in Table 1 were used as reference for a comparison of the dynamics of arsenic distribution by other routes of absorption.

Taking into consideration the distribution of arsenic in various tissues, we can observe the influence of the absorption route. In general, the content of arsenic in tissues is similar for the intravenous and intratracheal administration on the one hand and gastrointestinal and skin resorption on the other. The content of arsenic in tissues after 24 hr is less than 20% of the applied dose. The only excep-

Table 1. Dynamics of arsenic distribution after intravenous administration 1 mg/kg doses of Na_2HAsO_4 in rats.

Tissue or organ	Percentage of the dose/g tissue various times after administration					
	1 hr	2 hr	5 hr	24 hr	120 hr	240 hr
Intestines	0.15	0.14	0.072	0.035	0.092	0.055
Muscle and bones	0.13	0.10	0.066	0.053	0.055	0.051
Skin	0.20	0.10	0.039	0.044	0.053	0.038
Liver	3.85	3.73	3.47	2.75	2.17	1.73
Kidney	2.09	1.49	0.41	0.32	0.46	0.53
Spleen	1.60	1.96	2.15	1.72	1.90	1.70
Lung	2.72	2.47	2.49	2.00	1.85	1.09
Heart	0.47	0.32	0.23	0.28	0.40	0.30
Blood	1.49	2.16	2.78	2.73	3.95	3.76
Tail	0.83	0.79	0.73	0.64	0.24	0.17

tion is skin application, where over 50% of the dose is present outside blood, due to its deposit at the site of application. The results in Table 2 and 3 give the dynamics of arsenic distribution in liver and spleen is shown.

The dynamics of arsenic liver content is similar for intravenous and intratracheal administration but varies very much with skin resorption (Fig. 1). The first detected amounts, after 24 hr, are very small, but they increase in time. This is due to the relatively slow transport of arsenic from the site of application (the skin of the tail). The situation is similar if we consider the arsenic content of spleen. Here again we observe slower increases of arsenic

Table 2. Dynamics of arsenic distribution in liver with various routes of administration to rats.

Route of administration	Percentage of dose/g tissue at various times after administration					
	1 hr	2 hr	5 hr	24 hr	120 hr	240 hr
Intravenous	3.85	3.73	3.47	2.75	2.17	1.73
Intratracheal	2.49	2.59	3.25	3.08	1.69	1.02
Gastrointestinal	0.34	0.27	0.32	0.26	0.19	0.37
Skin resorption	—	—	—	0.066	0.36	0.28

Table 3. Dynamics of arsenic distribution in spleen with various routes of administration to rats.

Route of administration	Percentage of dose/g tissue at various times after administration					
	1 hr	2 hr	5 hr	24 hr	120 hr	240 hr
Intravenous	1.60	1.96	2.15	1.72	1.90	1.70
Intratracheal	0.71	1.07	1.55	1.69	2.48	2.75
Gastrointestinal	0.19	0.40	0.65	1.07	0.78	1.20
Skin resorption	—	—	—	0.29	0.63	0.96

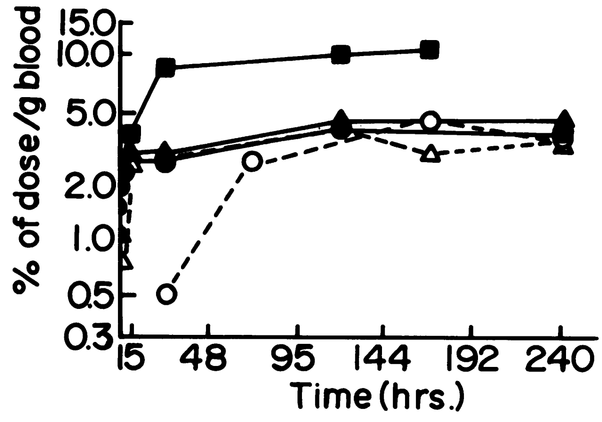

FIGURE 1. Absorption of arsenic in red cells *in vitro* and *in vivo* with administration by various routes: (×) *in vitro;* (▲) intratracheal; (o) skin resorption; (•) intravenous; (Δ) gastrointestinal.

concentrations after skin or gastrointestinal administration in comparison with the intravenous route.

Skin resorption first causes accumulation of arsenic in the skin and next slow, but continuous transport into the blood stream and to other tissues.

Skin resorption of arsenic from aqueous solutions of its salts was very substantial and for 0.01–0.2M concentrations was as much as 1.14–33.1 $\mu g/cm^2$-hr. Simple calculations considering 700 cm^2 to be the surface area of both hands show that skin resorption amounts to 0.8 to 23.2 mg/hr, which is considerably more than by absorption by the respiratory track in industrial conditions.

Arsenic is eliminated from the body in various ways: urine, feces, milk, respiratory tract, and skin. The largest amounts are eliminated in urine and feces. Elimination by the respiratory tract was negligible, amounting to 0.053–0.35% of the applied dose. In the same period of time the elimination by urine and feces was very substantial, as shown in Table 4.

Table 4. Elimination of arsenic in urine and feces at 10 days after various routes of administration.

Route of administration	Elimination of the absorbed dose, %	
	Urine	Feces
Intravenous	39	5
Intratracheal	35	15
Gastrointestinal	20	30
Skin	30	30

The elimination followed three-phase kinetics after intravenous and intratracheal application and a two-phase after gastrointestinal and skin resorption. The half-time of elimination was 2.5 hr in the first, about 10 hr in the second, and about 690 hr in the third phase. With skin resorption the half-time of elimination in urine was 28 hr in the first (second) and 240 hr in the second (third) phase. We can assume that after skin resorption the first phase does not exist.

On administration of repeated doses, the elimination in urine reaches a constant percentage of the absorbed daily dose. This is very important for the calculation of the absorbed amounts of arsenic.

The application of trivalent arsenic gives substantially the same results.

Figures 2 and 3 show arsenic elimination in urine and in feces. As to illustrate the most important differences, skin resorption and intravenous administration of the same applied dose were selected. The lines on the graphs indicate that processes of elimination of arsenic by both urine and feces are multiphase. The urine/feces elimination ratio changes with the route of administration,

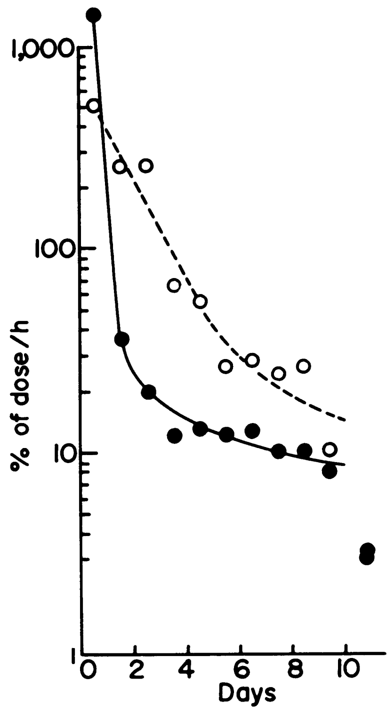

FIGURE 2. Elimination of arsenic in urine: (•) intravenous administration, dose 200 μg As, 38.53% of dose eliminated; (○) skin resorption, dose 203 μg As (by immersion in 0.1*M* solution), 30.39% of dose eliminated.

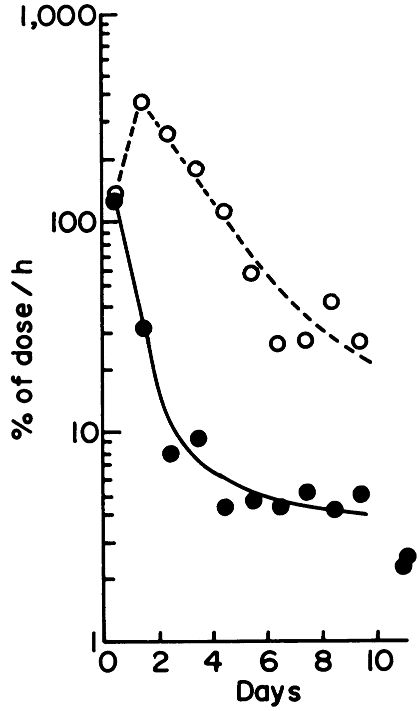

FIGURE 3. Elimination of arsenic in feces: (•) intravenous administration, dose 200 μg, 4.86% of dose eliminated; (○) skin resorption, dose 203 μg As (by immersion in 0.1*M* solution), 29.64% of dose eliminated.

which is not unique for arsenic and can be demonstrated for many other metals (Table 5).

The data in Table 5 (*6*) show that the urine/feces ratio of elimination of various metals varies very much for different routes of administration, which is very unfortunate for the purpose of developing integral exposure tests. Those tests are based mostly on determination of metals in urine. The concentrations of metals in urine are dependent on the mode of absorption, which is usually not known in either industrial or environmental conditions. This is a very strong obstacle to development of exposure tests, which are limited to only one route of absorption.

Table 5. Urine/feces ratio of elimination of various metals admistered intravenously, and by skin application.

Element	Route of administration	Urine/feces ratio
As	Intravenous	8 : 1
	Skin	1 : 1
Be	Intravenous	3 : 2
	Skin	1 : 9
Cr	Intravenous	1 : 1
	Skin	3 : 7
Hg	Intravenous	4 : 3
	Skin	2 : 5
Se	Intravenous	4 : 1
	Skin	2 : 1

Arsenic–Selenium Interaction

Many possible accelerators of arsenic elimination were investigated; among others selenium ammoniate was applied in single daily dose 0.2 mg/kg during the slow phase of arsenic elimination.

The selection of selenium salts was done on the basis of previous experiments by Dutkiewicz and Balcerska (7) in which selenium elimination in urine was accelerated after administration of arsenic salts. The rate of elimination was about 12% in the slow phase, in comparison with about 5% in the control group. Sodium arsenate was applied 2 times daily in single doses, 0.2 mg/kg, beginning from the third day of experiment. The most effective of the applied agents was glutathione, which gave an improvement in elimination of about 17%.

The application of selenium as an accelerating agent caused a 20%–30% increase in the elimination of arsenic in urine in the slow phase, the most effective agents being unithiol, penicylamine, methionine, and cysteine, giving about 100% increase in elimination.

Conclusions

Independent of the route of administration *in vivo* or in experiments *in vitro* considerable amounts of arsenic are bound to red blood cells.

The concentration of arsenic in blood is proportional to the absorbed dose in the range 0.1–4 mg As/kg.

The amounts of arsenic elimination in urine are considerable. The concentrations in urine are correlated with the absorbed dose.

Daily administration of the same dose leads to a constant level of arsenic elimination in urine.

The found relations could serve as baselines for the development of exposure indicators in humans.

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